ELECTROPORATION OF *Citrobacter* sp. WITH
PGAMDH 1301A UNLIMITED PLASMID

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ELECTROPORATION OF *Chlorella* sp. WITH pCAMBIA 1304 LINEARIZED PLASMID

By

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JABATAN SAINS BIOLOGI
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RESEARCH REPORT VERIFICATION

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: Electroporation of *Chlorella* sp. with pCAMBIA 1304 linearized plasmid oleh Oh Hong Yew, no. matrik: UK 9653 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains (Sains Biologi), Fakulti Sains dan Teknologi,Universiti Malaysia Terengganu.

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LIST OF ABBREVIATIONS

Bp	Basepair
BBM	Bold's Basal Medium
cDNA	Complimentary DNA
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Triphophates
E.coli	Escherichia coli
EDTA	Ethylene Diamine Tetraacetic Acid
Kb	Kilobase
Kv	Kilo Volts
LB	Luria-Bertanni
$MgCl_2$	Magnesium Chloride
$NaCl_2$	Sodium Chloride
OD	Optical Density
TAE	Tris Acetate EDTA

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ABSTRACT

Electroporation is a widely used method in plant transformation since it is very efficient. Transformation of pCAMBIA 1304 construct into microalgae, *Chlorella* sp. can play an important role as a model of fundamental studies of fatty acid biosynthesis pathways of *Chlorella* sp. As the initial step, the pCAMBIA 1304 plasmid was successfully extracted from *Escherichia coli* culture. The purity of the plasmid was 1.76 and the concentration was 0.55 µg/mL. The results showed the extracted plasmid was in high quality and can be further used in subsequent steps. The pCAMBIA 1304 plasmid was successfully verified by PCR technique with primers combination 35S-F/35S-R, GG-F/GG-R and 35S-F/GG-R. The sizes of amplified bands with were 326 bp, 676 bp and 1,426 bp respectively. PCR amplification of the CaMV 35S promoter and *gfp:uidA* genes fusion confirmed that the presence of pCAMBIA 1304 in extracted plasmid. The pCAMBIA 1304 plasmid was successfully linearized with *Eco*RI and purified by Wizard SV Gel and PCR Clean Up System Kit (Promega). The suitable voltage for electroporation of *Chlorella* sp. was determined with p35S-AP plasmid. The Agr mode (2.2 kV) was selected from six different electrical voltages used in electroporation of *Chlorella* sp. The *Chlorella* sp. cells were successfully electroporated with linearized pCAMBIA 1304 plasmid at 2.2 kV in 0.1 cm cuvette. The overnight electroporated *Chlorella* sp. cells were plated on BBM hygromycin (10 µg/mL) plate for selection of transformed cells. A total of 16 putative transformed colonies were randomly selected and transferred to BBM grid plates that containing 10 µg/mL.

ELEKTROPORASI *Chlorella* sp. DENGAN PLASMID pCAMBAI 1304 LINEAR

ABSTRAK

Elektroporasi adalah kaedah yang digunakan secara meluas dalam transformasi tumbuhan kerana keadah ini amat berkesan. Transformasi konstruk pCAMBIA 1304 ke dalam mikro-algae, *Chlorella* sp. boleh memainkan peranan yang penting sebagai satu model untuk asas pembelajaran laluan bio-sintesis asid lemak dalam *Chlorella* sp. Sebagai langkah permulaan, plasmid pCAMBIA 1304 telah berjaya diekstrakkan daripada kultur *Escherichia coli*. Ketulenan plasmid adalah 1.76 manakala kepekatan adalah 0.55 µg/mL. Keputusan ini menunjukkan plasmid yang diekstrakkan adalah berkualiti tinggi. Plasmid pCAMBIA 1304 telah berjaya dikesahkan dengan teknik PCR dan kombinasi primer 35S-F/35S-R, GG-F/GG-R dan 35S-F/GG-R. Saiz jalur-jalur adalah 326 bp, 676 bp dan 1,426 bp. Amplikasi PCR untuk promoter CaMV 35S dan gabungan gen-gen *gfp:uidA* telah memastikan kehadiran pCAMBIA 1304 dalam plasmid yang diekstrakkan. Plasmid pCAMBIA 1304 berjaya dihadamkan dengan DNA selitan, *EcoR*I dan ditulenkan dengan "Wizard SV Gel andR Clean-Up System Kit (Promega)". Plasmid p35S-AP digunakan untuk memilih voltan yang sesuai. Mod Agr (2.2 kV) telah dipilih daripada enam voltan elektrik yang berlainan untuk digunakan dalam elektroporasi *Chlorella* sp. Sel-sel *Chlorella* sp. telah berjaya dielektroporasikan dengan plasmid pCAMBIA 1304 linear pada 2.2 kV dalam kuvet 0.1 cm. Sel-sel yang telah dielektroporasi dan dikultur semalaman berjaya disebar atas media BBM dengan 10 µg/mL "hygromycin" dan koloni-koloni yang berjaya ditransformasikan kelihatan. Sebanyak 16 koloni dipilih secara rawak dan dipindahkan ke atas media BBM (grid plate) yang mengandungi 10 µg/mL "hygromycin".